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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Christophe de ROMEUF

Title: TREATMENT OF PATHOLOGIES WHICH ESCAPE THE IMMUNE RESPONSE, USING OPTIMIZED ANTIBODIES

Appl. No.: 10/527,666

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TRANSMITTAL

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

Submitted herewith are the English translation of the specification of French priority no. 02 11415 filed on September 13, 2002 and a Declaration verifying that the English translation filed with the USPTO is a true and correct translation of the priority document.

The Commissioner is hereby authorized to charge any additional fees which may be required regarding this application under 37 C.F.R. §§ 1.16-1.17, or credit any overpayment, to Deposit Account No. 19-0741. Should no proper payment be enclosed herewith, as by the credit card payment form being unsigned, providing incorrect information resulting in a rejected credit card transaction, or even entirely missing, the Commissioner is authorized to charge the unpaid amount to Deposit Account No. 19-0741.

Respectfully submitted,

Date May 11, 2006

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application Serial N° : 10/527,666

Filed : September 15, 2003

For : Treatment of pathologies which escape the immune response, using optimized antibodies

DECLARATION

I, Nicolas Torno, c/o Cabinet Regimbeau, 20 rue de Chazelles, 75017 Paris (France), hereby declare that I am well acquainted with the French and English languages and hereby certify that to the best of my knowledge and belief the following is a true translation of French priority n° 02 11415 filed on September 13, 2002.

All statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true; and further, these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any document or any registration resulting therefrom.

Date : March 17, 2006

Nicolas TORNO

The present invention relates to the use of an antibody for preparing a medicament intended to induce the secretion of at least one cytokine by an effector cell belonging to the immune system, said antibody being characterized in that it can be obtained by means of a method of selection comprising bringing CD16 receptor-expressing effector cells of the immune system, which may or may not be transformed, into contact in a reaction medium in the presence of the test antibody and of the antigen for said antibody, and measuring the amount of at least one cytokine produced by the CD16 receptor-expressing cell.

Immunotherapy by means of polyclonal or monoclonal antibodies is in the process of becoming one of the most important aspects of medicine. On the other hand, the results obtained in clinical trials appear to be contrasting. In fact, the monoclonal antibody may prove to be insufficiently effective. Today, research is directed toward the immunoglobulin Fcγ fragment in order to improve antibody properties. In the end, this should make it possible to obtain antibodies which interact with and activate the receptors of effector cells (macrophage, T lymphocyte and NK cell).

Binding of antibodies on its ligand can induce activation of effector cells via Fc receptors. One of the consequences of activation is non only induction of functional properties such as ADCC or complement activation but also cytokines porduction. these cytokines produced at the activation site of effectors can have different biological activities.

In connection with the invention, we found that activation of receptors in effector cells produces very

different responses leading to the release of several cytokines. These cytokines are responsible for activation or inhibition of components of the immune system depending of the case.

Thus, the problem is to known what is the ability of a given antibody to stimulate cytokines production by effector cells and what are the consequences of such activation depending on the nature of the released cytokines.

For example, it may occur that an antibody directed against a given antigen be completely inefficient when it is produced in mouse myeloma cell lines whereas it is shown to be very efficient when produced in other cell lines.

The objective is therefore to use antibodies that have been selected before for their ability to activate such or such components of the immune system, for example ADCC or on the contrary such as they are not able to induce a cytotoxic response.

Indeed, we found that binding of an antibody to its ligand can induce Jurkat CD16 transfected cells which induces IL-2 secretion. A strong correlation is observed between IL-2 secretion by Jurkat CD16 and the ADCC activity mediated by CD16 of effector cells.

The invention therefore proposes the use of antibodies selected using a Jurkat CD16 test, by measuring secreted IL-2 or other cytokines, which makes it possible to guarantee the biological activity of said antibodies for therapeutic use.

Description

Thus, in a first aspect, the present invention relates to the use of an antibody for preparing a medicament

intended to induce the secretion of at least one cytokine by an effector cell belonging to the immune system, said antibody being characterized in that it can be obtained by means of a method of selection comprising bringing CD16 receptor-expressing effector cells of the immune system, which may or may not be transformed, into contact in a reaction medium in the presence of the test antibody and of the antigen for said antibody, and measuring the amount of at least one cytokine produced by the CD16 receptor-expressing cell.

The term "transformed cell" is intended to mean a cell that has been genetically modified so as to express a receptor, in particular the CD16 receptor.

Preferably, for selecting the antibodies, a Jurkat line transfected with an expression vector encoding the CD16 receptor is used as effector cell. This cell line is particularly advantageous since it is immortalized et grow indefinitely in culture medium.

Said released cytokines are interleukins, interferons and tissue necrosis factors (TNF). Thus, the antibody selected has the ability to induce the secretion of at least one cytokine chosen from IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, etc., TNFα and IFNγ by the CD16 receptor-expressing effector cells of the immune system.

Preferably, the antibody selected has the ability to induce the secretion of IL-2 by the CD16 receptor-expressing effector cells of the immune system. The amount of interleukin IL2 secreted reflects the quality of the antibody bound by the CD16 receptor as regards its antigen-binding integrity (Fc function) and effectiveness (antigenic site). The measurement of the amount of IL2 is correlated with an ADCC-type activity.

Besides, the antibody can be selected after being

purified.

The selection can take place on antibodies produced by cells commonly used for the production of therapeutic antibodies, such as CHO, YB2/0, human lymphoblastoid cells, insect cells and murine myeloma cells. The selection may also be applied to the evaluation of antibodies produced by transgenic plants or transgenic mammals.

In a particular embodiment, the antibody selected is capable of inducing the secretion of at least one cytokine by a leukocytic cell, in particular of the NK (natural killer) family, or by cells of the monocyte-macrophage group.

The invention also relates to the use of the selected antibodies described above that are specific for an antigen which originates from a pathological cell or from an organism that is pathogenic for humans.

This antibody is a monoclonal or polyclonal antibody.

For example, the antibody is a monoclonal or polyclonal antibody having anti-human red blood cell Rhesus specificity.

The antibody according to the invention may also be an antibody directed against viruses that are pathogenic for humans, against malignant tumor antigens or against the antigens of a bacterium or of a parasite that is pathogenic for humans.

Advantageously, the antibody selected shows an increase of more than 100%, 250%, 500% or 1000% in the amount of IL-2 release compared with the control in the absence of antibody or in the presence of a given antibody as negative reference. The method described above can optionally be carried out in the presence of human

immunoglobulins (IVIgs).

In a supplementary aspect, the invention is directed toward the use of said selected antibodies as a therapeutic support in human medicine, in particular for producing a medicinal product intended for the treatment of autoimmune and inflammatory diseases, cancers and infections with pathogenic agents.

In still another embodiment, the invention also relates to a kit for evaluating the biological activity of an antibody, comprising means and reagents required for the dosage of at least one cytokine, for example IL-2, IFN and/or TNF, and effector cells expressing one or several FcR receptors, such as receptor CD16.

Legends

Figure 1: Release of cytokine (IL-2, IFN and TNF) by leukocytes that are antibody-activated in the presence of their target

A- Leukocyte activation scheme
B- The leukocytes were incubated with various antibodies in the presence of red blood cells. After an overnight incubation period, the release of TNFa and IFNγ into the supernatant was quantified by ELISA.

Figure 2: Release of cytokine (IFN, TNF) by NK cells that are antibody-activated in the presence of their target (LFB-R297-RBC) - 06/13/02

A- NK cell activation scheme.
B- Purified NK cells were mixed with various anti-D antibodies in the presence of Rhesus + red blood cells. After an overnight incubation period, the release of TNFα and of IFNγ into the supernatant was quantified by ELISA.

Figure 3: Release of cytokine (IFN, TNF) by NK cells that are antibody-activated in the presence of their target (LFB-R297-RBC) - 08/13/02

A- NK cell activation scheme.
B- Purified NK cells were mixed with various anti-D antibodies in the presence of Rhesus + red blood cells. After an overnight incubation period, the release of TNFα and of IFNγ into the supernatant was quantified by ELISA.

Figure 4: Release of IL2 by Jurkat CD16 activated by an anti-CD20

A- Jurkat cell activation scheme.
B- Jurkat CD16 cells were mixed with various anti-CD20 antibodies (murine antibody CAT13 and chimeric antibody C273) in the presence of Raji cells and of PMA. After an overnight incubation period, the release of IL-2 into the supernatant was quantified by ELISA.

Figure 5: Release of IL2 by Jurkat CD16 activated by an anti-D

A- Jurkat cell activation scheme.
B- Jurkat CD16 cells were mixed with various anti-D antibodies in the presence of Rhesus + red blood cells and of PMA. After an overnight incubation period, the release of IL-2 into the supernatant was quantified by ELISA. DF5 expressed in YB2/0 and T125 expressed in CHO Lec13 induce strong secretion of IL2.

Example 1: Jurkat CD16 assay

control Antibodies:

WinRho polyclonal antibodies, DF5-EBV monoclonal antibody, DF5-YB2/0 monoclonal antibody.

Principle:

This assay evaluates the ability of the anti-D antibodies to bind to the CD16 receptor (Fc gamma RIII) expressed on Jurkat CD16 cells, and to induce IL2 secretion.

This assay consists in bringing the following into contact in a 96-well plate: the anti-D antibodies, the papain-treated Rhesus-positive red blood cells, the Jurkat CD16 cells and PMA.

After an overnight incubation period at 37°C, the P96 are centrifuged and the amount of secreted IL2 is assayed in the supernatant.

Operating protocol

Material

Positive control antibodies: Poly-D WinRho, DF5-YB2/0.

Negative control antibody: DF5.

Rhesus-positive red blood cells.

Jurkat CD16 cells.

IL2 assay kit: Quantikine from R/D.

Method

Treatment of red blood cells with papain.

1 ml of pellet of red blood cells diluted in PBS is incubated with 1 ml of a papain solution (1 mg/ml) for 10 min at 37°C. Three washes are then carried out in H_2O-0.15M NaCl.

Reaction mixture:

-Antibody: 50 µl of a dilution to 150 ng/ml in IMDM 5% SVF,

-PMA: 50 µl of a dilution to 40 ng/ml in IMDM 5% SVF,

-red blood cells treated with papain. 50 µl at 8×10^6/ml in IMDM 5% SVF,

-Jurkat CD16. 50 µl at 2×10^6/ml in IMDM 5% SVF.

Overnight incubation at 37°C.

Then, centrifugation of the plates, removal of 100 µl of supernatants and assaying of IL2 with the commercial kit. Reading at 450 nm.

The values (in pg/ml) are given in the form of a histogram for each sample.

Example 2: Activation of NK cells and production of IL2 and of IFNγ

Study model: NK cells purified from peripheral blood. Applications: enhancement of an anti-tumor response. IL2 induces activation of T lymphocytes and of the NK cells themselves, which can go as far as stimulation of cell proliferation. IFNγ stimulates the activity of CTLs and can enhance the activity of macrophages.

Example 3: Activation of monocyte-macrophages and production of TNF and of IL-1Ra

Applications: Enhancement of phagocytosis and induction of anti-inflammatory properties. TNF stimulates the proliferation of tumor-infiltrating lymphocytes and macrophages. IL-1Ra is a cytokine produced by macrophages which compete with IL1 at the level of its receptor and thus exerts an anti-inflammatory effect.

Example 4: Activation of dendritic cells and production of IL10

Applications: Induction of tolerance specific to certain antigens. IL10 is a molecule that inhibits the activation of various effector cells and the production of cytokines.

CLAIMS

1. Use of an antibody for preparing a medicament intended to induce the secretion of at least one cytokine by an effector cell belonging to the immune system, said antibody being characterized in that it can be obtained by means of a method of selection comprising bringing CD16 receptor-expressing effector cells of the immune system, which may or may not be transformed, into contact in a reaction medium in the presence of the test antibody and of the antigen for said antibody, and measuring the amount of at least one cytokine produced by the CD16 receptor-expressing cell.

2. The use according to claim 1, wherein the method of selection is performed with a Jurkat expressing the CD16 receptor.

3. The use according to one of claims 1 to 2, wherein released cytokines are interleukins.

4. The use according to one of claims 1 to 2, wherein released cytokines are interferons.

5. The use according to one of claims 1 to 2, wherein released cytokines are Tissue Necrosis Factors (TNF).

6. The use according to one of claims 1 to 2, wherein the antibody selected has the ability to induce the secretion of at least one cytokine chosen from IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, TNFα and IFNγ by the CD16 receptor-expressing effector cells of the immune system.

7. The use according to one of claims 1 to 2, wherein the antibody selected has the ability to induce the secretion of IL-2 by the CD16 receptor-expressing

effector cells of the immune system.

8. The use according to one of claims 1 to 7, wherein the antibody is selected after being purified.

9. The use according to one of claims 1 to 8, wherein the antigen originates from a pathological cell or from a pathogenic organism for humans.

10. The use according to one of claims 1 to 9, wherein the antibody is selected for its ability to induce secretion of at least one cytokine by a leukocyte cell, in particular of the NK family (Natural Killer) or by cells of the monocytes-macrophages group.

11. The use according to one of claims 1 to 10, wherein the antibody is a monoclonal or polyclonal antibody.

12. The use according to one of claims 1 to 11, wherein the antibody has anti-human red blood cell Rhesus specificity.

13. The use according to one of claims 1 to 11, wherein the antibody is directed against viruses that are pathogenic for humans.

14. The use according to one of claims 1 to 11, wherein, the antibody is directed against malignant tumor antigens.

15. The use according to one of claims 1 to 11, wherein the antibody is directed against the antigens of a bacterium or of a parasite that is pathogenic for humans.

16. The use according to one of claims 1 to 15 wherein, the selected antibody shows an increase of more than 100%, 250%, 500% or 1000% in the amount of IL-2 release compared with the control in the absence of antibody or

in the presence of a given antibody as negative reference.

17. The use according to one of claims 1 to 16 wherein, as a therapeutic support in human medicine.

18. The use according to one of claims 1 to 17 for manufacturing a medicinal product intended for the treatment of autoimmune and inflammatory diseases, cancers and infections with pathogenic agents.

19. A kit for evaluating the biological activity of an antibody, comprising means and reagents required for the dosage of at least one cytokine, for example IL-2, IFN and/or TNF, and effector cells expressing the CD16 receptor.

Activation of leukocytes by anti-D antibodies

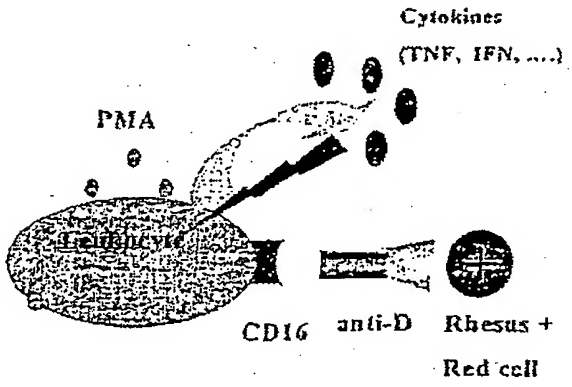

Figure 1A

Release of cytokines (IL2, IFN and TNF) by leukocytes that are antibody-activated in the presence of their target

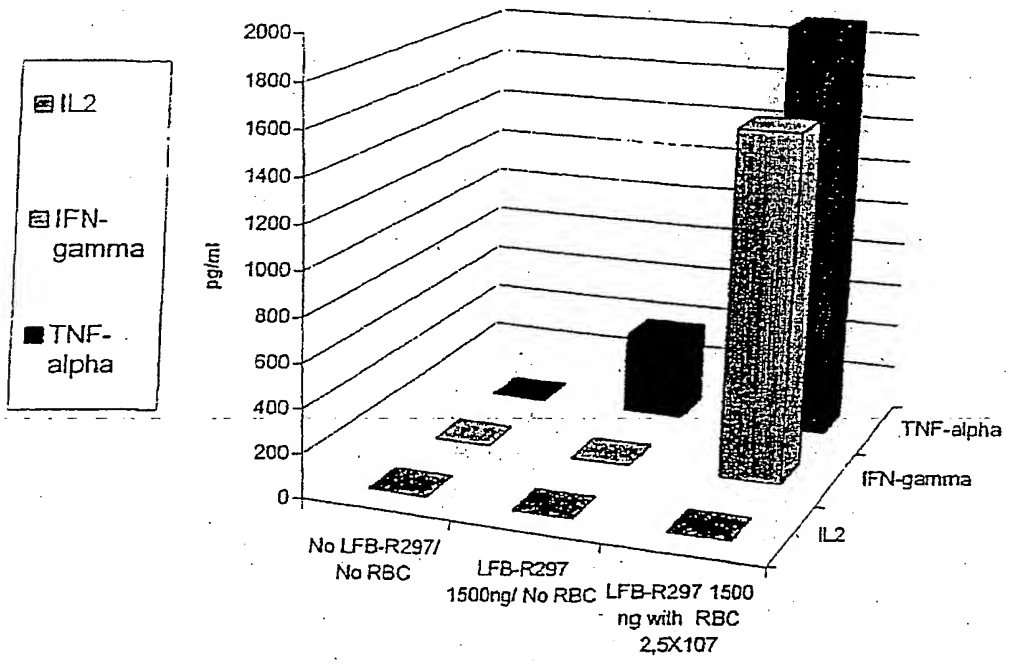

Figure 1B

Induction of cytokine (IFN, TNF) secretion by NK cells activated by anti-D antibodies and their target (LFB-R297-RBC)

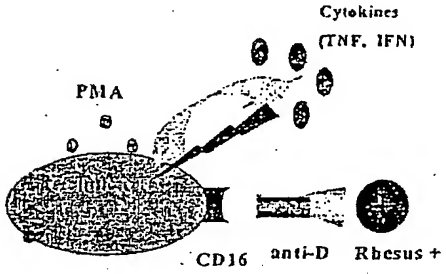

Figure 2A

Release of cytokines (IFN, TNF) by NK cells that are antibody-activated in the presence of their target (LFB-R297-RBC)

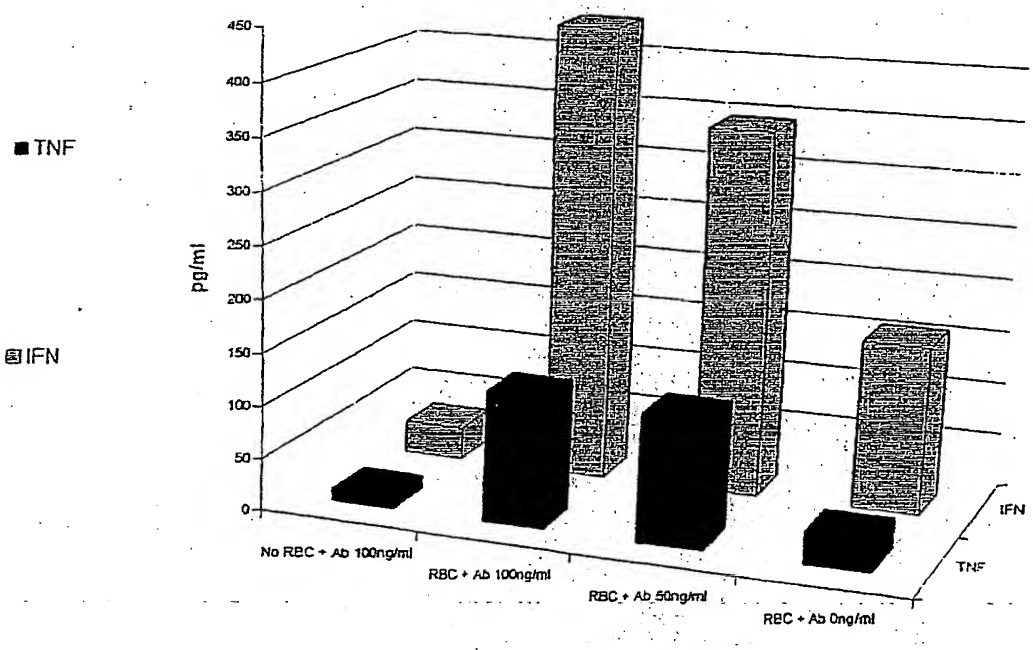

Figure 2B

Secretion of IL2 by Jurkat CD16 activated by the anti-CD20 CAT 13 or C273

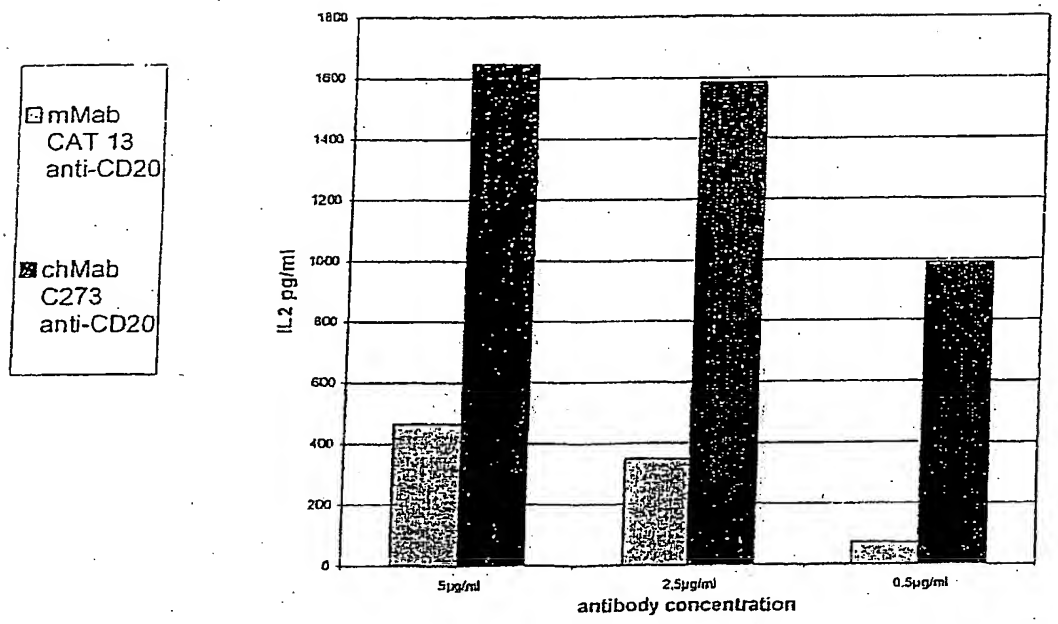

Figure 3

Release of IL2 from Jurkat CD16, induced by anti-D antibodies

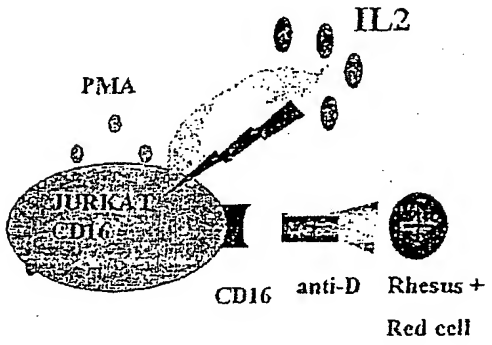

Figure 4A

Secretion of IL2 by Jurkat CD16 activated by various anti-Rhesus D antibodies

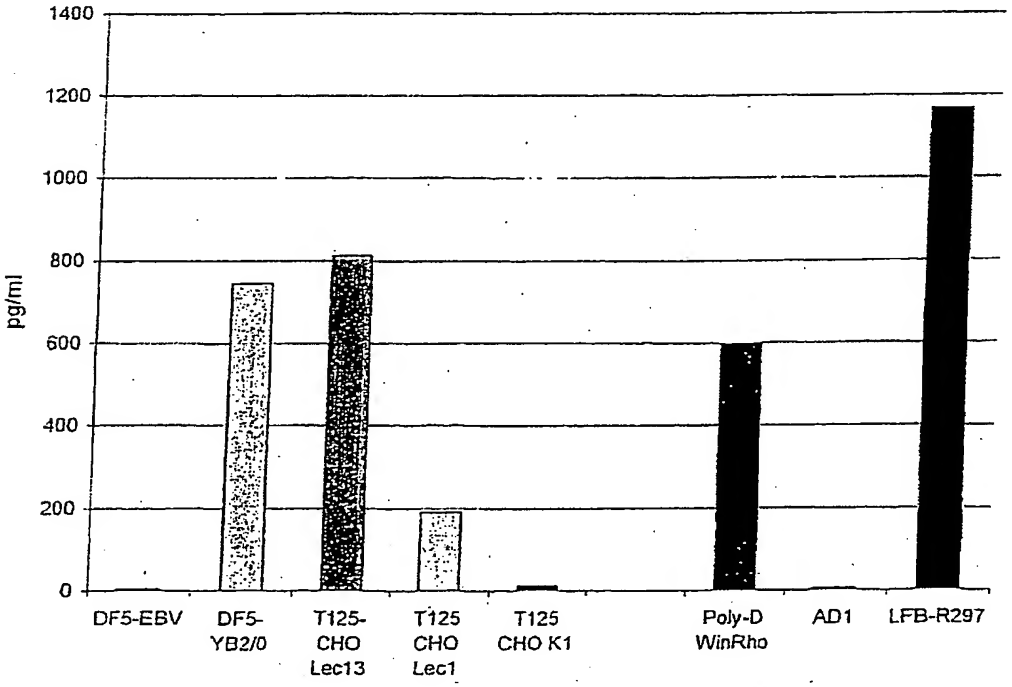

Figure 4B